



Artificial Lymphocytosis in Tuberculosis

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COLORADO SPRINGS, COLO.



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ARTIFICIAL LYMPHOCYTOSIS IN TUBERCULOSIS

BY GERALD BERTRAM WEBB, M.D., WM. WHITRIDGE WILLIAMS,
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At the 1909 meeting of this Association a paper¹ was read, entitled "Some Hematological Studies in Tuberculosis," which contained details of a change in the differential blood-picture brought about by altitude, which had not before been noted.

This change consisted in an increase of lymphocytes, and is well seen in the following chart.

Dr. Basinger, who joined us in the present work, arrived in Colorado Springs (6000 feet) in August, 1909, and at once essayed to show, by his own blood examinations, that our observations might be wrong.

It is especially to be noted in this chart that the large lymphocytes increased from 170 per c.mm. on arrival to a permanent height of 2000 per c.mm., as it is this cell which would appear to have the power of destroying the tubercle bacillus.

The chart also illustrates well the biologic phenomenon of overproduction, which we find to be constant in our blood counts during the first two weeks of residence here of a healthy adult from sea-level.*

The total lymphocytes increased from 2400 on arrival to 4200 at the end of two weeks, and then dropped to 3800, where they have since remained.

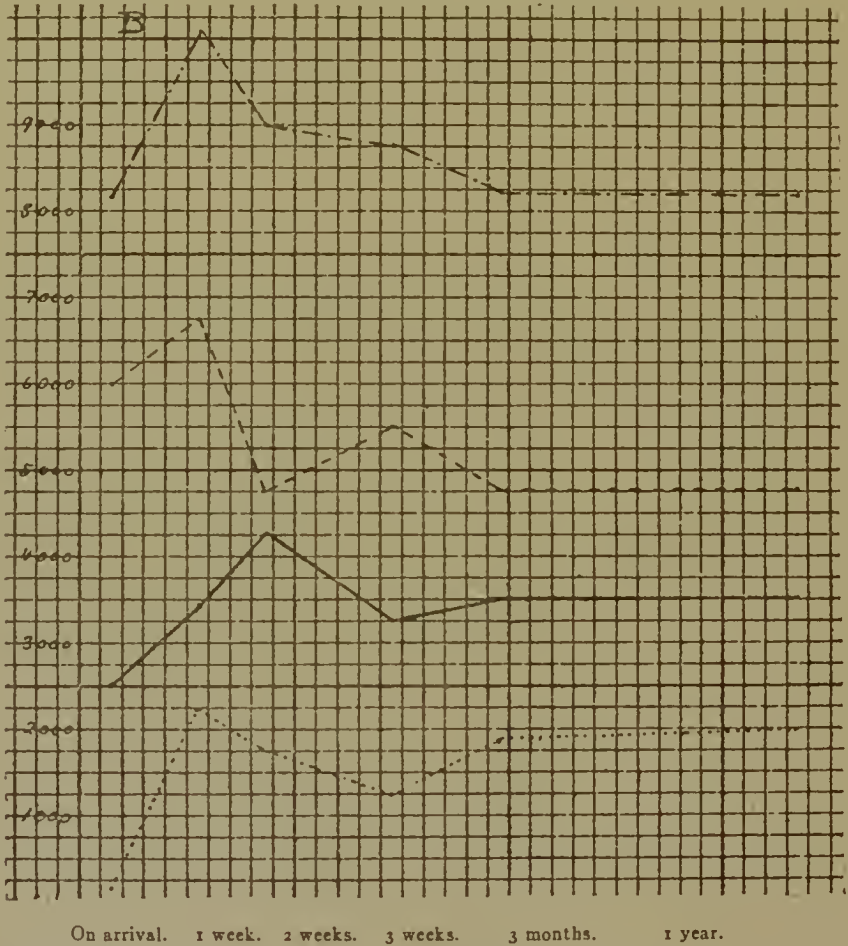
Reference to our former paper¹ will show why the lymphocyte is considered of such importance in the defense against tuberculosis, and consequently why we consider the increase in these cells brought about by altitude as being of such importance in the cure of tuberculous invalids and in the high degree of immunity our residents possess to this disease. We reason that the same factor which causes the increase of the red corpuscles in altitude must also

* Dr. Henry Sewall,² of Denver, some years ago pointed out that a characteristic effect of high altitude upon the circulation is an overloading pressure of the right side of the heart. This may have a bearing on the marrow hyperplasia of altitude.

originate the increase of the lymphocytes, namely, marrow hyperplasia, as shown by Züntz.³

Josué⁴ shows that in localized experimental tuberculosis in rabbits the bone-marrow responds to infection by a very intense proliferation of the

The accompanying chart records his observations



Explanation of Chart I. - - - - - Total leukocytes per c.mm. - - - - - Total polymorphonuclears per c.mm. ——— Total mononuclears per c.mm. Large lymphocytes per c.mm.

mononuclear leukocytes, and also that the subcutaneous injection of tuberculin will bring about similar marrow activity.

He states that a comparable phenomenon occurs in man. It can, therefore, be considered that by removing a suitable tuberculous invalid to an

altitude, nature is being aided by the stimulation given to the lymphoid marrow tissue.

In addition to evidence referred to¹ as to the defensive behavior of lymphocytes, Daels⁵ has shown that the von Pirquet skin reaction to tuberculin is characterized by the collection of lymphoid cells at the irritated area.

Cadbury⁶ found in patients dying of pulmonary tuberculosis that if the bone-marrow was found proliferated, it was practically the rule to find a considerable number of lymphocytes.

It is especially noteworthy that during the past year Bergel,⁷ in Germany, and Marie and Fiessinger,⁸ in France, have made the important discovery that the lymphocytes contain a ferment of lipolytic power capable of splitting wax and fat into glycerin and fatty acids. Following Opie's method of nomenclature, we would suggest for this ferment the name of lympholipase. It has long been known that at least 30 per cent. by weight of the body of the tubercle bacillus is composed of waxy substances, and the chronicity of tuberculosis has been thought to be due to the difficulty the defensive mechanism of the host must have to destroy this waxy substance. The importance, therefore, of the discovery of the increase in lymphocytes due to altitude is enhanced by the discovery of lympholipase.

Pribram⁹ believes in a lipolytic ferment being present in the blood, but does not feel sure, on account of his failure in a small number of experiments, that it is yet completely proved that lipase originates in the lymphocytes.*

We have ourselves studied the lysis of tubercle bacilli by the lymphocytes of cockroaches and grasshoppers. These insects would seem to have entirely this type of cell, corresponding to our large lymphocytes.

Injections of tubercle bacilli can readily be made into one hind leg, and at various intervals lymphocytes containing tubercle bacilli in all stages of digestion can be found by withdrawing some circulating fluid from the other hind leg.

We have been struck by the length of time necessary for this digestion—often several days and not several hours only, as might be thought.

* As this paper goes to press an excellent article appears by Fiessinger,¹⁵ on the part played by lipase in the defense against tuberculosis. Plates are also given which illustrate the lysis of tubercle bacilli by the lymphocytes of the bee moth larva.

Fiessinger concludes that the lymphocytes furnish lipase, which destroys the waxy parts of the tubercle bacillus, and that it is then necessary for the polymorphonuclear leukocytes to appear with their leukoprotease, which only works in an alkaline medium, to complete the digestion.

He would seem to overlook the fact that the lympholipase, by splitting wax into fatty acid and glycerin, might supply the acid medium in which the lymphoprotease could act.

Then, too, we know that complete lysis can proceed in insects which have no polymorphonuclear leukocytes, but only lymphocytes.

It has been shown by Opie¹⁰ that the injection of living leukocytes, consisting of an approximately equal suspension of polymorphonuclear and of mononuclear cells, obtained from normal dogs and injected into dogs which have been artificially infected with the tubercle bacillus, can inhibit the development of tuberculous lesions. This would seem to show the value of increasing the numbers of leukocytes.

Following our observations on the effect of altitude in increasing the lymphocytes it was now argued that if bone-marrow hyperplasia provoked the increase of mononuclears reported, then artificial hyperemia of accessible bones might yield the same result. To this end a rubber bandage was applied as a tourniquet to the legs, as high up the thighs as possible, with sufficient pressure to interfere with the return venous flow, but not enough to obstruct the arterial.

The following chart represents the results of the first application of this method to man:

NORMALS. ONE HOUR

	TOTAL LEUKOCYTES			PERCENTAGE MONONUCLEARS			TOTAL MONONUCLEARS			TOTAL POLYMORPHONUCLEARS			PERCENTAGE OF CHANGE			
	Before.	During.	After.	B	D	A	B	D	A	B	D	A	Mono-nuclears		Polymorphonuclears	
I.	6684	7372	14784	30.0	40.5	55.0	2607	3428	8131	4010	3007	6570	+30	+211	-2.5	+115
II.	9000	11280	14550	30.5	43.5	41.0	3011	4907	5068	5040	6317	8588	+25	+52	+6	+45
III.	5400	..	8280	51.5	..	01.0	2811	..	5050	2457	..	3220	..	+70	..	+31
IV.	11076	11208	15504	42.0	44.0	50.0	4052	4912	7752	0002	5884	6077	+6	+60	-3	+14
V.	7620	8400	11844	50.5	41.0	40.0	3848	3453	5874	3620	4758	5507	-9	+50	+31	+53
VI.	5976	..	5526	53.0	..	52.0	3107	..	2874	2800	..	2052	..	-0	..	-5.5

NORMALS. ONE-HALF HOUR

VII.	9816	..	12168	37.5	..	30.5	3681	..	4906	5030	..	7118	..	+30	..	+20
VIII.	11472	..	16416	38.5	..	33.0	4407	..	5417	6482	..	10588	..	+23	..	+63
IX.	5040	..	5676	61.5	..	51.0	3100	..	2895	1940	..	2753	..	-7	..	-42

TUBERCULOUS

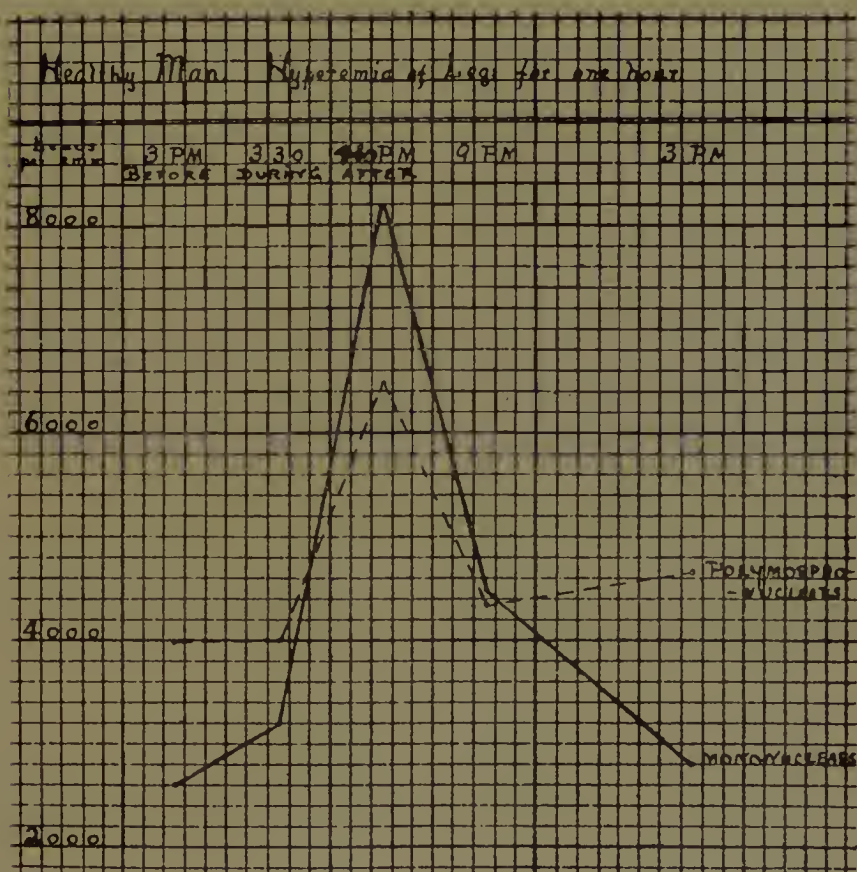
X.	7602	7560	10560	40.0	41.5	41.5	3077	3137	4382	4061	4082	5640	+2	+42	-18	+14
XI.	15372	..	15768	23.0	..	27.5	3536	..	4336	11836	..	11432	..	+23	..	-3
XII.	10176	..	10036	36.0	..	37.0	3053	..	4046	6258	..	6835	..	+10	..	+0
XIII.	7440	..	9600	36.0	..	34.0	2678	..	3264	4724	..	6240	..	+22	..	+32
XIV.	14136	..	18204	21.5	..	37.5	3039	..	6820	10743	..	11286	..	+125	..	+5

I and IV, bandage removed at end of fifty minutes, due to patient complaining of faintness, nausea, and failing vision. V, forty minutes, nausea and faintness; VI, fifty minutes; IX, twenty-five minutes, throbbing in legs; X, fifty-three minutes, nausea; XI, forty-five minutes; XII, forty minutes; XIII, thirty minutes; XIV, twenty-three minutes, nausea and vomiting.

The results so obtained are an average total of mononuclear increase of 75 per cent. in normal individuals after one hour's application, and 18 per cent. increase of mononuclears after one-half hour treatment. The mononuclear increase is much more marked than the polymorphonuclear. Two nucleated red corpuscles (normoblasts) were observed in the blood slide from case 1.

The subjective symptoms are: A swelling of the legs, the skin becoming reddened or even bluish, and a feeling of drowsiness in most cases; local discomfort is not marked, but some numbness and tingling occur.

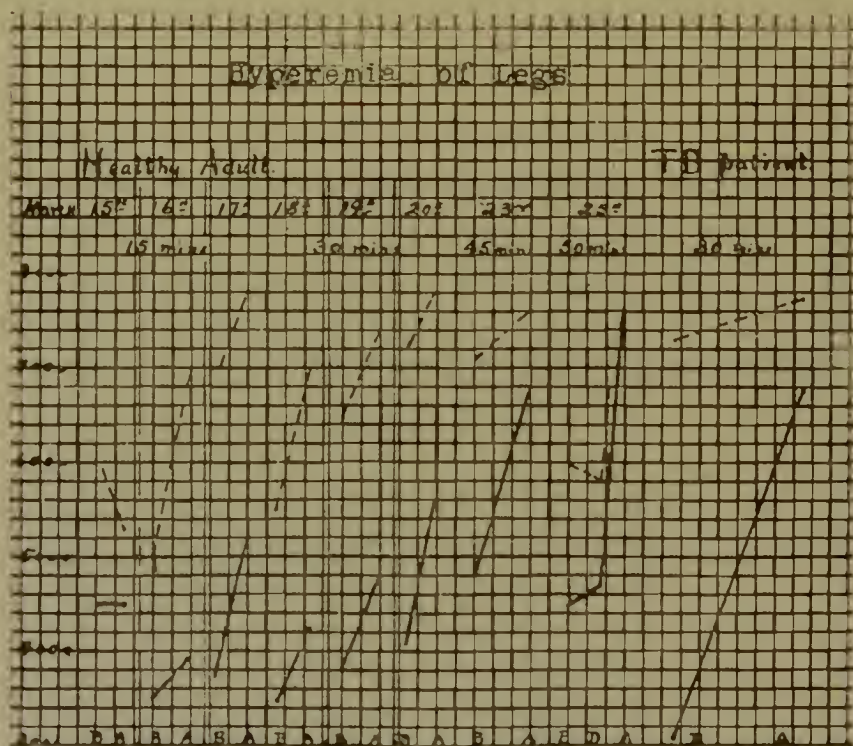
The following charts illustrate graphically such experiments:



The question naturally arises, can the marrow-cells so supplied with excess of blood actually multiply in this length of time?

Carnegie Dickson¹¹ states that mitosis is a comparatively rapid process in the marrow, and it is for this reason that even in proliferating marrow karyokinetic figures are rare.

Dickson, in replying to a letter asking how long a time would be necessary for marrow-cells to divide, writes: "With regard to your query *re* cell division,



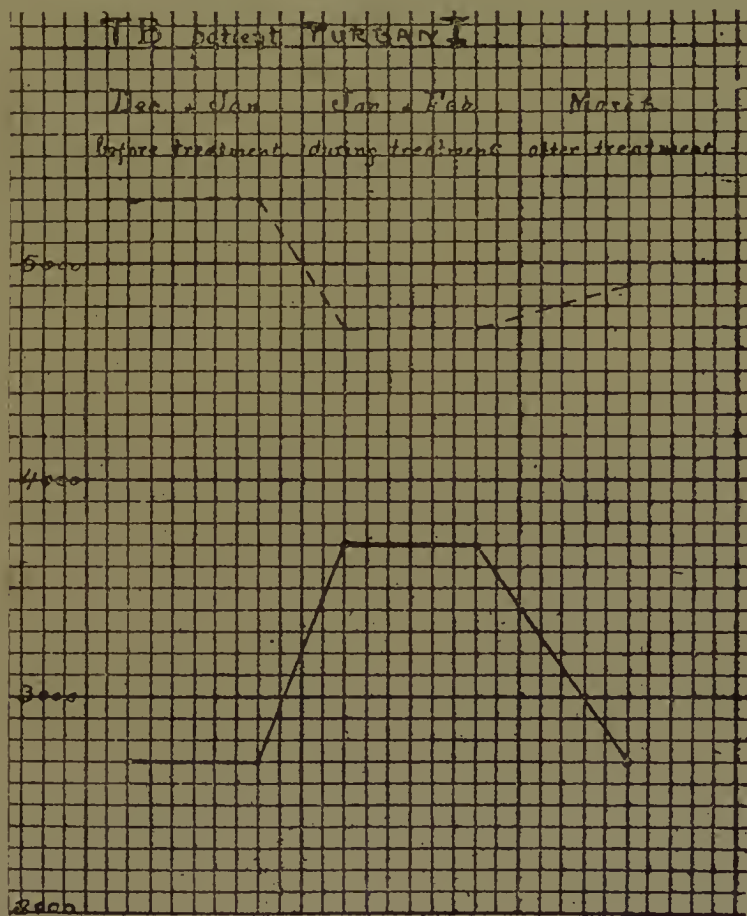
B = before hyperemia of legs.
A = after hyperemia of legs.

I should think that cell division can certainly occur within the limits of time you mention (fifteen minutes to an hour), although I have no direct evidence to prove it, yet I think that the indirect evidence on the subject renders the statement a fairly safe one.

Ross and Macalister,¹² in an article "On Cancer Research," have made interesting observations on lymphocytes. They state that these cells contain no Altmann granules in their cytoplasm, as do the polymorphonuclear leuko-

cytes. The lymphocytes contain, however, granules arranged on the walls of their nuclei, which give the chromatin reaction with certain stains.

By using a jelly considered to possess a reproductive excitant, mitosis of lymphocytes was discovered, and the granules mentioned collected around the equator of the now spindled-shaped nucleus and divided into sixteen portions, which appeared as chromosomes.



During January and February the cough and expectoration ceased, and six pounds in weight were gained.

Mitosis of a lymphocyte would appear, from the experiments described, to take place in a few minutes.

The authors are inclined to believe that lymphocytes are reproductive, and, therefore, remarkably different from the polymorphonuclear leukocytes.

We have frequently seen in blood-smears following the artificial marrow hyperemia large lymphocytes with two or three segments in the nuclei and others cell distinctly multinucleated.

The changes in the blood-picture do not take place in a few minutes, as would be expected by a mere flushing out, and the greatest results obtained have been after from one-half hour to an hour.

In an address on artificial lymphocytosis before the Denver County Medical Society in April, 1910, it was stated that lymphocytosis could also be produced by constant hyperemia of accessible long bones, as the following observations attest.

These patients applied to the upper arms, day and night, garter elastic, one inch wide, which was just tight enough to leave an indentation in the skin after removal. They suffered no discomfort, neither did the arms visibly swell.

Mr. M.—Cured pulmonary tuberculosis.

	POLYMORPHO- NUCLEARS	SMALL LYM- PHOCYTES	LARGE LYM- PHOCYTES	LARGE MONO- NUCLEARS	TRANSI- TIONALS	TOTAL PER CENT. LYM- PHOCYTES	EOSINOPHILES	MAST CELLS	TOTAL LEU- KOCYTES
At start.....	61.0	8.0	20.0	2.0	..	(39)	
After one week.....	51.0	6.5	38.5	2.5	1.0	(47.5)	1.0	0.5	
After two weeks.....	32.0	18.5	48.0	1.0	..	(67.5)	..	0.5	
Hyperemia omitted one week.....	57.5	16.5	16.5	6.5	2.5	(42)	..	0.5	

Mrs. R.—Pulmonary tuberculosis. Turban III.

At start.....	69.5	1.0	28.0	1.0	..	(30.0)	0.5	..	
After five days.....	52.5	7.5	38.0	1.0	..	(46.5)	..	1.0	
After ten days.....	49.5	9.5	38.5	2.0	..	(50.0)	0.5	..	
Hyperemia omitted for one week.....	62.5	12.0	21.5	2.5	1.5	(37.5)	

Mr. S.—Pulmonary tuberculosis. Turban III.

Two months before.....	61.5	13.0	20.5	4.0	..	(37.5)	1.0	..	8,500
At start.....	70.0	9.5	17.5	1.5	0.5	(29.0)	0.5	5.0	12,000
Continued arm hyperemia:									
One week.....	42.0	9.5	44.0	3.0	0.5	(57.0)	0.5	0.5	10,000
Two weeks.....	49.5	6.0	40.0	2.5	1.0	(48.5)	1.0	..	15,000
Three weeks.....	49.0	7.0	42.0	1.0	0.5	(50.5)	..	0.5	15,000
Four weeks.....	51.0	8.0	36.0	2.5	0.5	(47.0)	1.0	1.0	12,000
Five weeks.....	56.0	10.0	43.0	0.5	..	(53.5)	0.5	..	
Arms omitted, hyperemia of one leg this week....	56.5	14.0	18.5	6.0	3.5	(42.0)	0.5	1.0	
Arm hyperemia again this week.....	50.5	12.0	30.0	4.5	1.5	(48.0)	1.0	0.5	
Two weeks.....	69.5	12.0	13.0	4.0	1.0	(30.0)	..	0.5	
Three weeks.....	48.0	9.5	32.5	5.5	4.5	(52.0)	
Four weeks.....	52.0	14.5	25.5	5.5	2.0	(47.5)	..	0.5	

Miss V. Turban III.

	POLYMORPHO-NUCLEARS	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES	LARGE MONO-NUCLEARS	TRANSITIONALS	TOTAL PER CENT. LYMPHOCYTES	EOSINOPHILES	MAST CELLS	TOTAL LEUKOCYTES
Before hyperemia.....	62.0	6.0	17.0	2.0	3.0	(38.0)	7,000
" "	70.5	7.0	15.0	4.0	1.5	(27.5)	1.5	1.0	14,000
" "	72.5	6.5	14.0	3.5	1.0	(25.0)	2.5	..	17,000
" "	72.0	7.5	14.0	3.5	2.5	(27.5)	5.0	..	16,000
After two weeks arm hyperemia.....	30.0	2.5	60.5	3.0	3.0	(69.0)	1.0	..	25,000
After six weeks.....	50.5	13.5	34.0	2.0	..	(49.5)	0.0	..	25,000
After seven weeks.....	72.0	0.5	19.5	1.0	..	(25.5)	2.5	..	16,000

It was not possible in all these tests to make total leukocyte counts.

We have previously shown the increase¹ in lymphocytes following Bier's hyperemia, and the following case illustrates this again:

Mr. H.—Tuberculous right knee. Bier's hyperemia applied to right leg thirty minutes daily.

	POLYMORPHO-NUCLEARS	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES	LARGE MONO-NUCLEARS	TRANSITIONALS	TOTAL PER CENT. LYMPHOCYTES	EOSINOPHILES	MAST CELLS	TOTAL LEUKOCYTES
At start.....	64.5	9.0	16.5	4.0	4.0	(33.5)	1.0	1.0	10,000
After one week.....	61.0	8.5	26.0	3.0	0.5	(38.0)	5.5	..	
After two weeks.....	48.0	10.0	35.5	4.5	1.0	(51.0)	0.5	0.5	
After three weeks.....	54.0	15.0	27.5	1.5	0.5	(44.5)	1.0	0.5	
After six weeks.....	52.0	13.5	28.5	3.0	1.5	(46.5)	1.5	..	
After nine weeks.....	59.0	8.5	28.5	1.0	1.0	(39.0)	2.0	..	10,000

It was next questioned what the results of a hot mustard foot-bath might be on the blood-picture; theoretically, it was expected similar change would take place. The following is the result of such an experiment on a healthy male adult.*

2.50 P.M.: Leukocytes, 9600; mononuclears, 3216; polymorphonuclears, 6192.

	POLYMORPHO-NUCLEARS	SMALL LYMPHOCYTES	LARGE LYMPHOCYTES	LARGE MONO-NUCLEARS	TRANSITIONALS	TOTAL PER CENT. LYMPHOCYTES	EOSINOPHILES	MAST CELLS	TOTAL LEUKOCYTES
	64.5	23.0	8.5	2.0	..	(33.5)	1.0	1.0	..

* The time-honored remedy of cod-liver oil has been shown to produce a digestion lymphocytosis.

3 P.M.: Feet placed in tub hot water kept at 55°-57° C., in which was some mustard, for 1 hour.
 4.30 P.M.: Leukocytes, 12,360; mononuclears, 4820; polymorphonuclears, 7292

	POLYMORPHO- NUCLEARS	SMALL LYM- PHOCYTES	LARGE LYM- PHOCYTES	LARGE MONO- NUCLEARS	TRANSI- TIONALS	TOTAL PER CENT. LYM- PHOCYTES	EOSINOPHILES	MAST CELLS	TOTAL LEU- KOCYTES
	59.0	28.5	6.5	3.0	1.0	(39.0)	1.0	1.0	..

Total leukocyte change = + 29 per cent.
 Total mononuclear change = + 50 per cent.
 Total polymorphonuclear change = + 18 per cent.

MARROW HYPEREMIA. EXPERIMENTS ON RABBITS— SERIES I.

Eighteen rabbits were divided into three groups. All rabbits were inoculated on the same day with 0.5 c.c. of standardized bovine tubercle suspension.

Rabbits 1 to 6 served as controls. Rabbits 7 to 12 received daily hyperemia of the hind legs for one hour. A thin rubber tubing was passed across the small of the back and brought around under the groin and along the inner side of the ischial tuberosities, and again to the small of the back, and clamped. We had shown by the following experiment on a normal rabbit that a leukocytosis and lymphocytosis could be produced.

3 P.M.: Leukocytes, 5940. per c.mm.; mononuclears, 4663 per c.mm.; polymorphonuclears, 1129 per c.mm.

3.15: Bandaged about hips.

4.15: Leukocytes, 9996; mononuclears, 7047; polymorphonuclears, 2499.

Total leukocyte increase = + 68 per cent.
 Total polymorphonuclear increase = + 127 "
 Total mononuclear increase = + 51 "

The rabbits were then kept at rest in a capacious box, and some would lie with their hind legs stretched out, while others remained squatting. It was found difficult to regulate the pressure accurately, and the rabbits would at times get into positions when the femoral artery could not be felt, and there is no doubt that on some days the marrow hyperemia was not so successful as on others.

Rabbits 13 to 18 received each one hour similar hyperemia of their hind legs immediately before the inoculation of the emulsion. They received no further treatment.

The 18 rabbits were kept under identical conditions; they were fed and watered daily at 8 o'clock, and bloods were always taken at 11 o'clock.

The blood examinations were made every third day, and the series 7 to 12, which will be spoken of as the hyperemia group, had the tourniquet applied immediately following the blood-taking.

Theobald Smith,¹³ inoculating rabbits into an ear vein with 0.5 c.c. of a

well-clouded suspension of tubercle bacilli in bouillon, killed rabbits weighing between 1300 and 2200 grams in seventeen to twenty-one days. We carefully followed his directions, and the density of our tubercle suspension was nearly equivalent to that of a bouillon culture of typhoid bacilli twenty to twenty-four hours old, and nearly as homogeneous.

In inoculating rabbits 1 to 6 a mistake was made in attempting to use a 10 c.c. syringe. This was found unwieldy, and resulted in rabbits 1, 2, 3, and 6 failing to get their full dose. In spite of this it will be noticed that rabbits 2 and 3 died in a comparatively short time.

In rabbit 4 chart, at the X mark, the left hind leg was found to be quite swollen, and in a condition of chronic hyperemia due to the development of a large groin abscess. This condition kept up for the remainder of the animal's life.

We are indebted to Dr. W. F. Martin, of Colorado Springs, for the report of a patient with pulmonary tuberculosis who showed considerable improvement following the development of a large fibroid uterus which caused venous stasis in the legs.

The culture of the bovine tubercle bacillus used in all our work was obtained through the courtesy of Dr. M. P. Ravenel from Dr. John Reichel, of the Pennsylvania State Live Stock Sanitary Board. It is known in their laboratory as Culture H, and was virulent for guinea-pigs, rabbits, dogs, goats, and cattle. The culture was grown on glycerin-agar.

The following averages for the 18 rabbits were found before the beginning of the experiments:

Average leukocyte count (18 rabbits)	9800 per c.mm.
Average rectal temperature.....	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; font-size: 3em; line-height: 1;">{</div> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; margin-right: 10px;">extremes.....</div> <div style="display: inline-block; vertical-align: middle;">6060 to 14630</div> </div> </div> <div style="display: inline-block; vertical-align: middle; margin-left: 10px;"> <div style="display: inline-block; vertical-align: middle; margin-right: 10px;">= 101.6° F.</div> <div style="display: inline-block; vertical-align: middle; margin-right: 10px;">{</div> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; margin-right: 10px;">extremes.....</div> <div style="display: inline-block; vertical-align: middle;">100.2° to 103.4° F.</div> </div> </div>
Average percentage mononuclears.....	69
	<div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; font-size: 3em; line-height: 1;">{</div> <div style="display: inline-block; vertical-align: middle;"> <div style="display: inline-block; vertical-align: middle; margin-right: 10px;">extremes.....</div> <div style="display: inline-block; vertical-align: middle;">54° to 82.5° F.</div> </div> </div>

It was not possible to obtain all males, and it was unfortunate that in the hyperemia group four females were included. These gave birth to litters, as indicated in the charts.

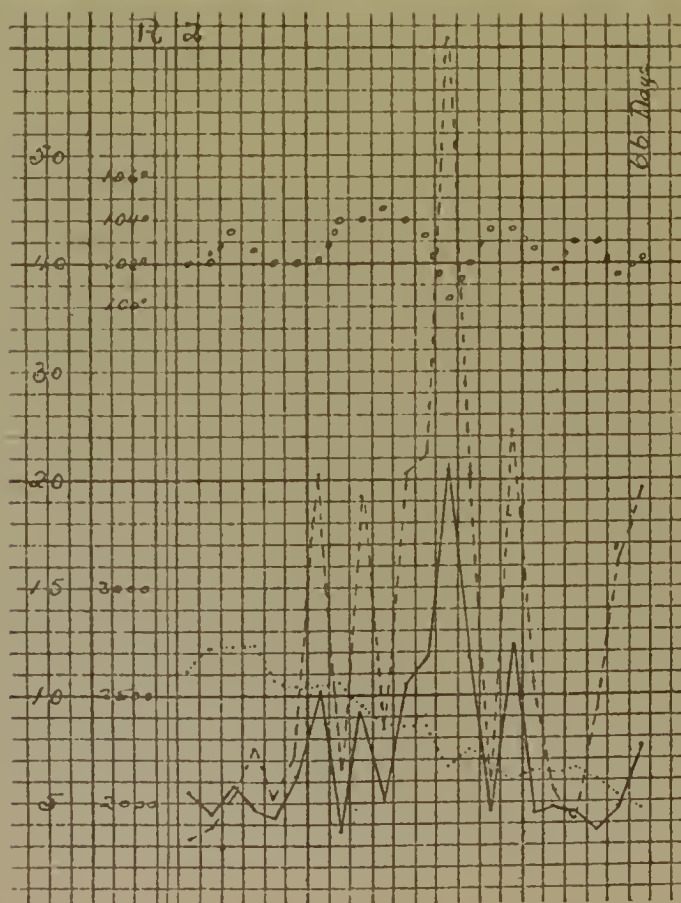
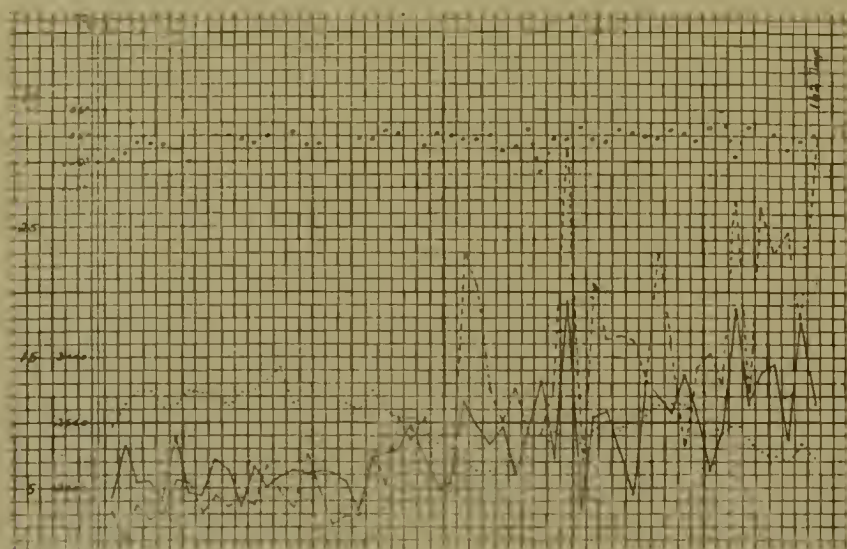
Rabbit 9 gave birth to young, as seen in chart, and this condition was followed by complete inversion and extrusion of the uterus. As this organ could not be replaced, the rabbit was sacrificed on the forty-fifth day.

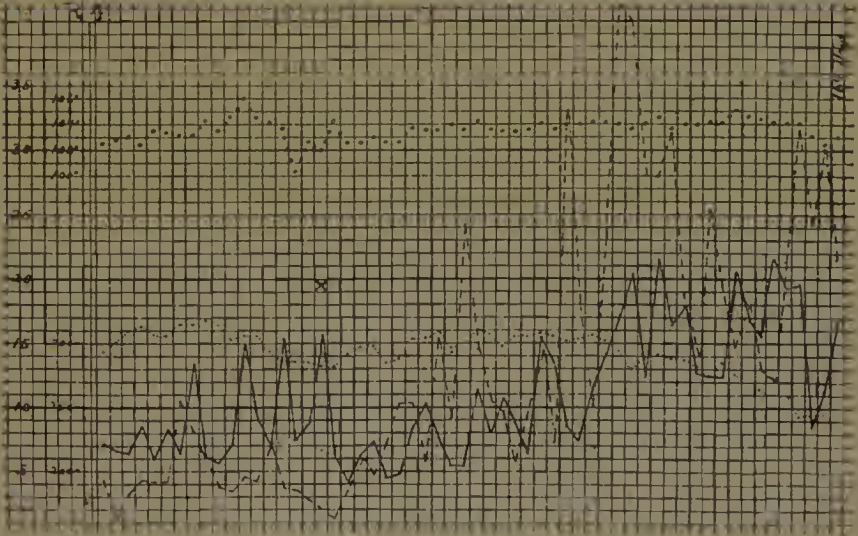
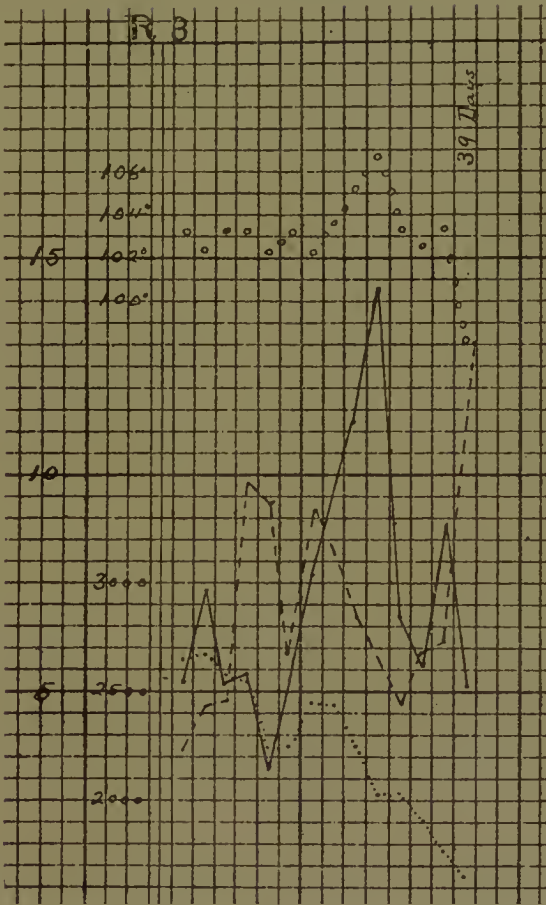
The marrow of normal rabbits was studied macroscopically before and after hyperemia, and of some of the infected rabbits at their death, but on account of the dark-red color already due to the hyperplasia induced by altitude, no difference in color could be detected.

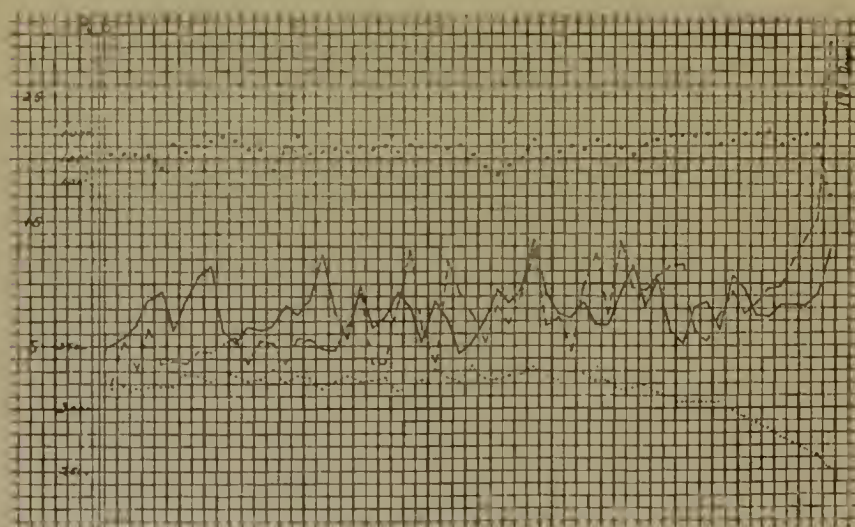
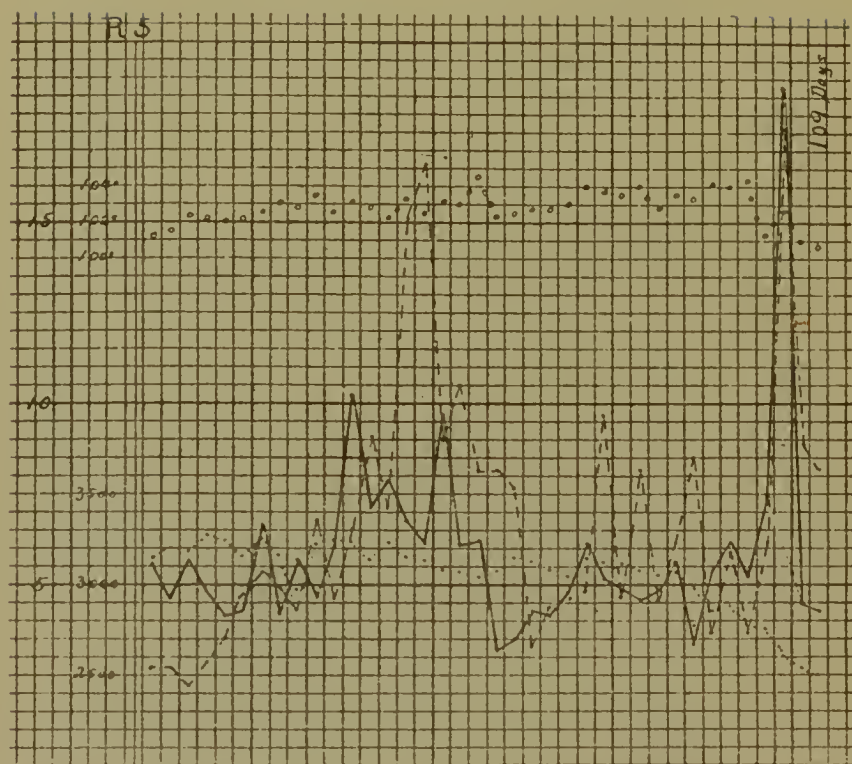
The marrow of rabbits at this altitude (6000 feet) is of a dark-red color, whereas at sea-level it is a grayish red⁴ (Josué).

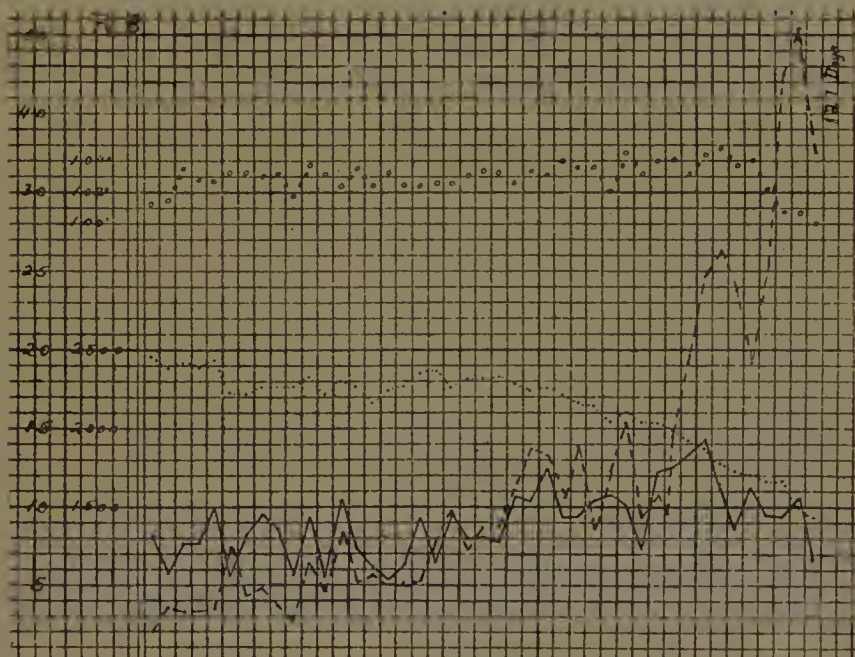
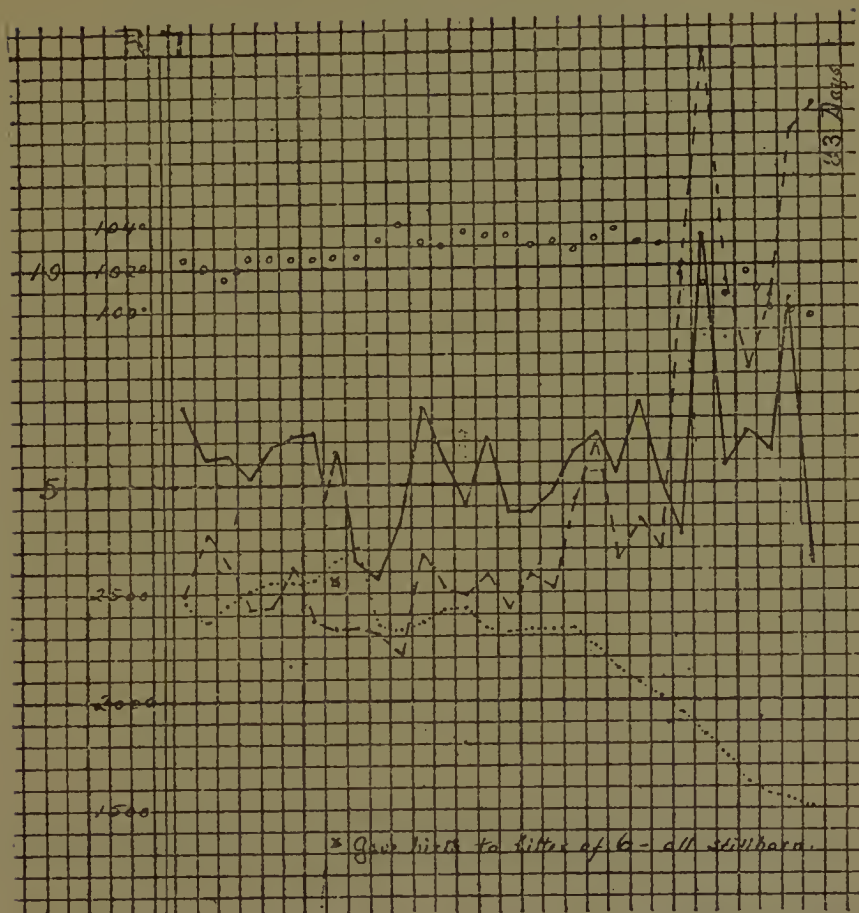
o o o o o o Temperature. ----- Polymorphonuclear leukocytes per c.mm.
 ----- Total mononuclear leukocytes per c.mm. Weight in grams.

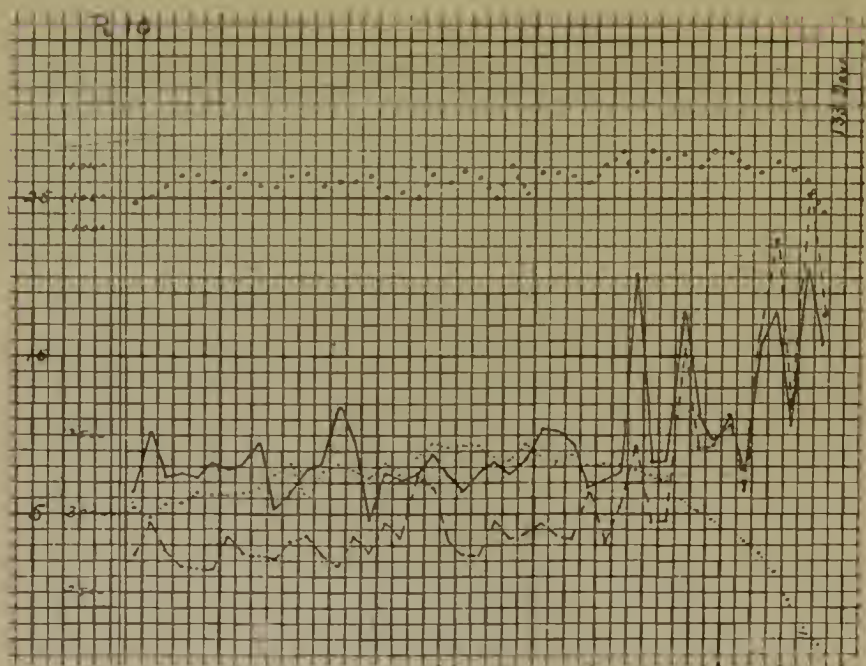
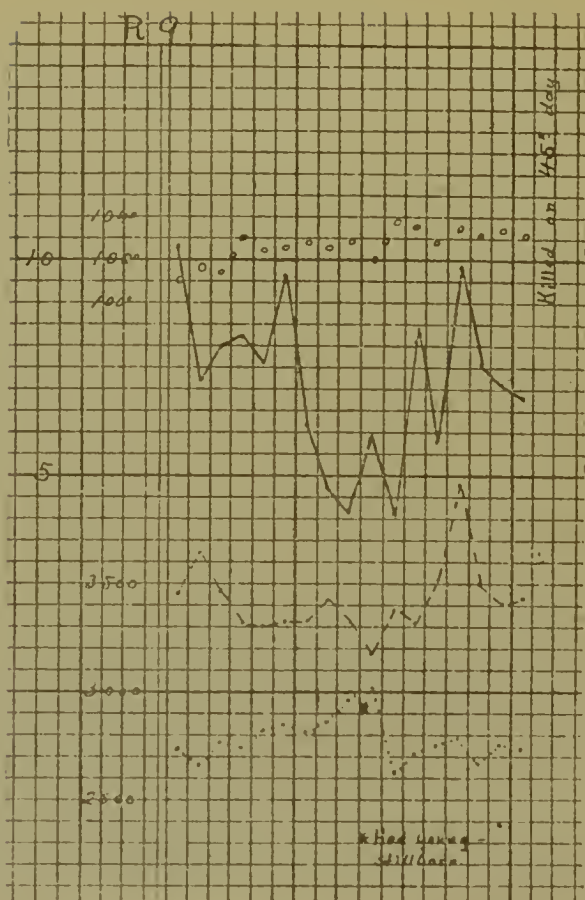
The left-hand column of figures represents thousands per c.mm. The second column of figures represents above temperature, below weight in grams.

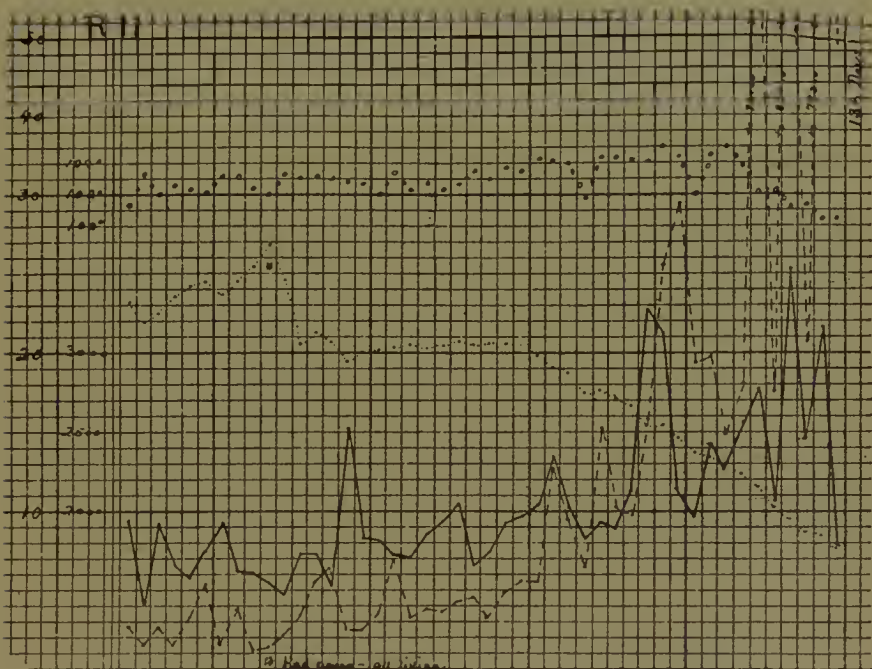


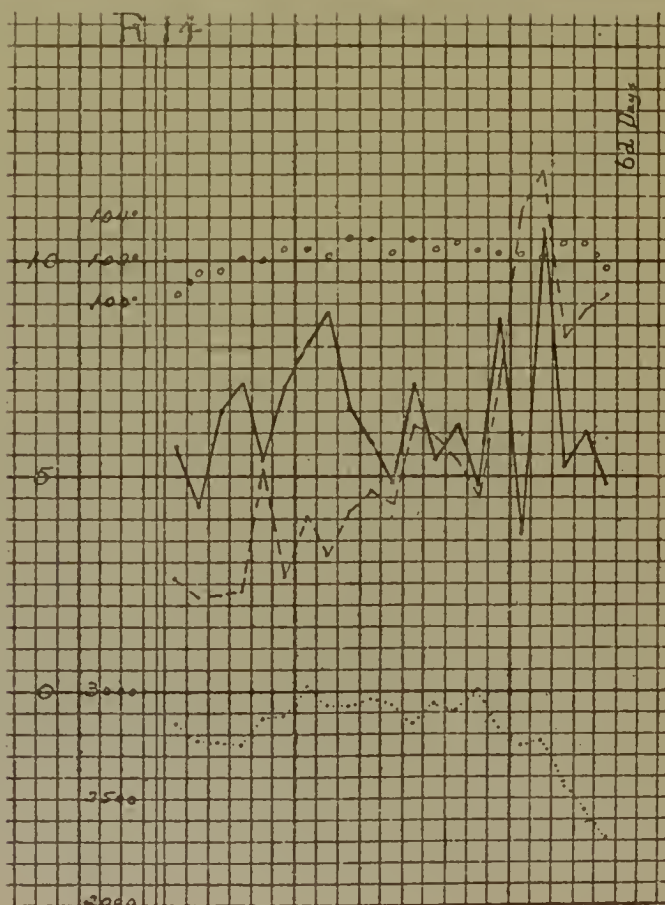
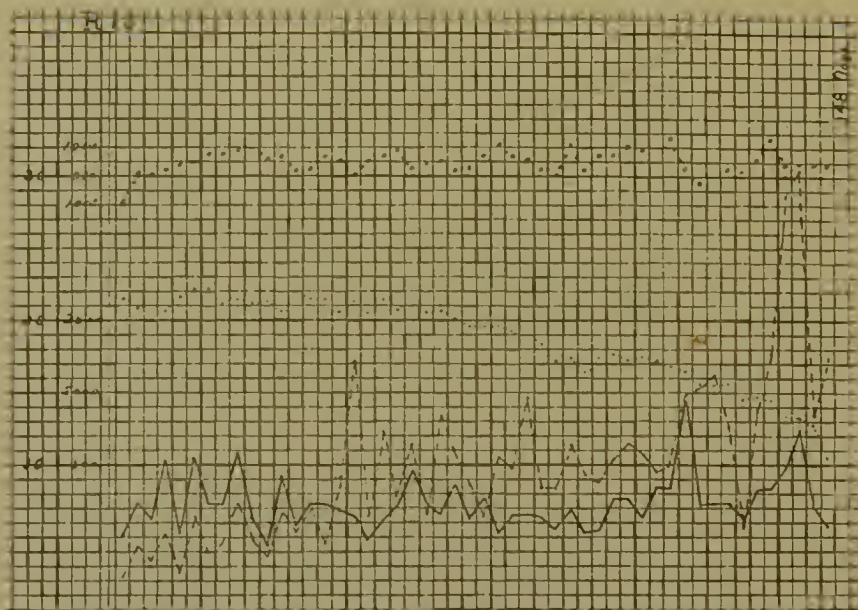


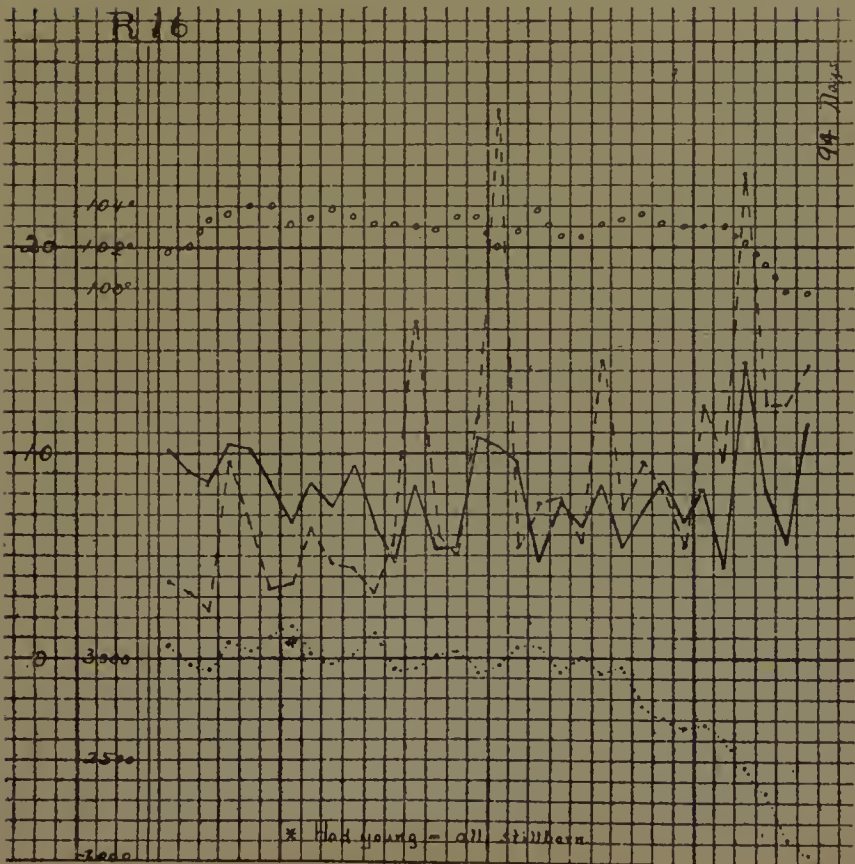
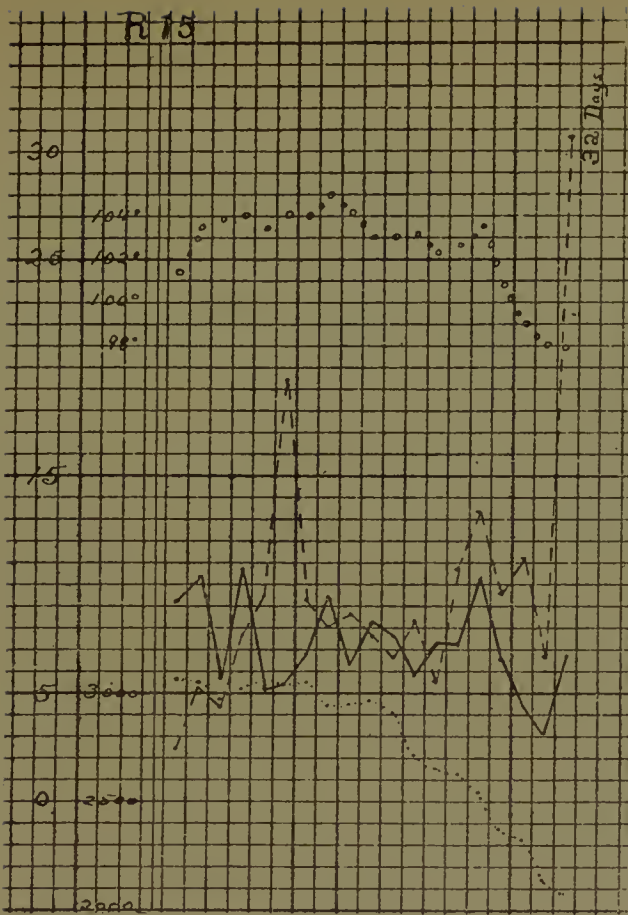


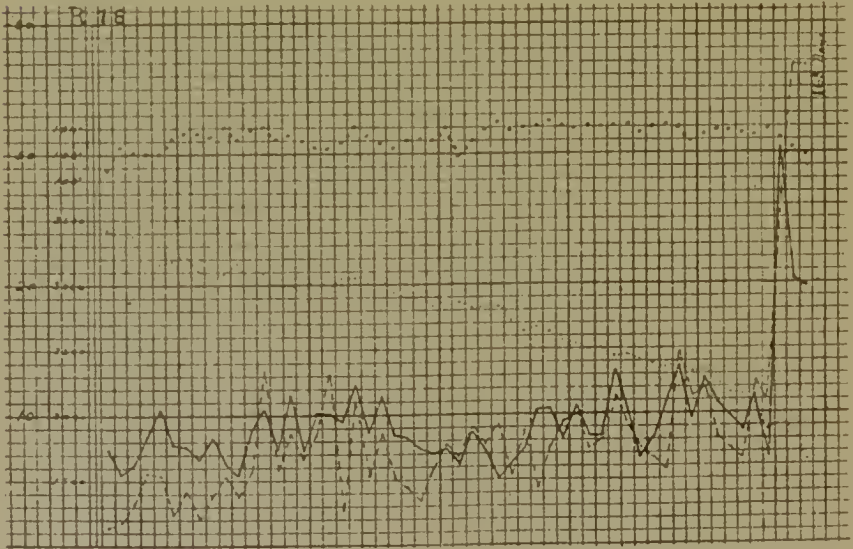
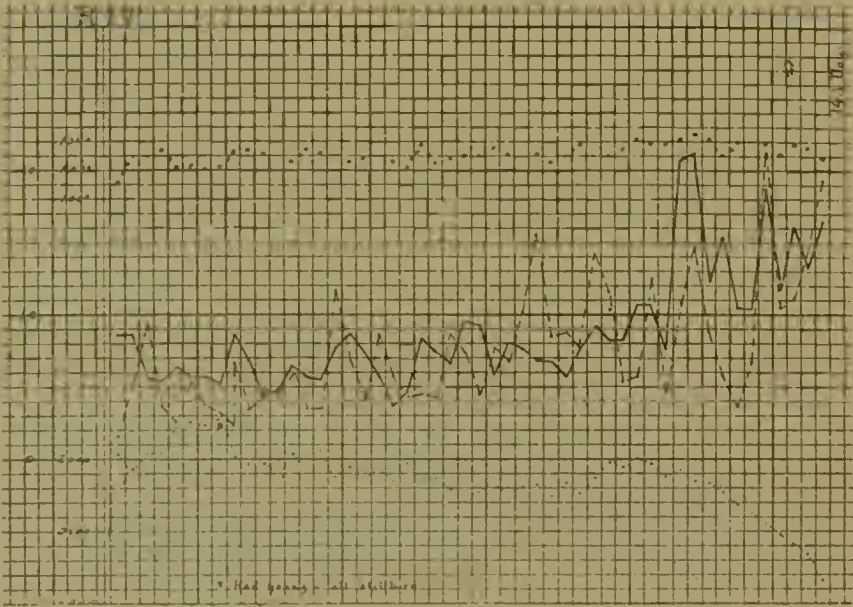












In surveying the eighteen charts, one important impression would seem to stand out in the hyperemia group, namely, the long-continued high level of the lymphocytes and the suppression, until quite late, of marked leukocytosis.

AUTOPSY RECORD OF RABBITS 1 TO 18.

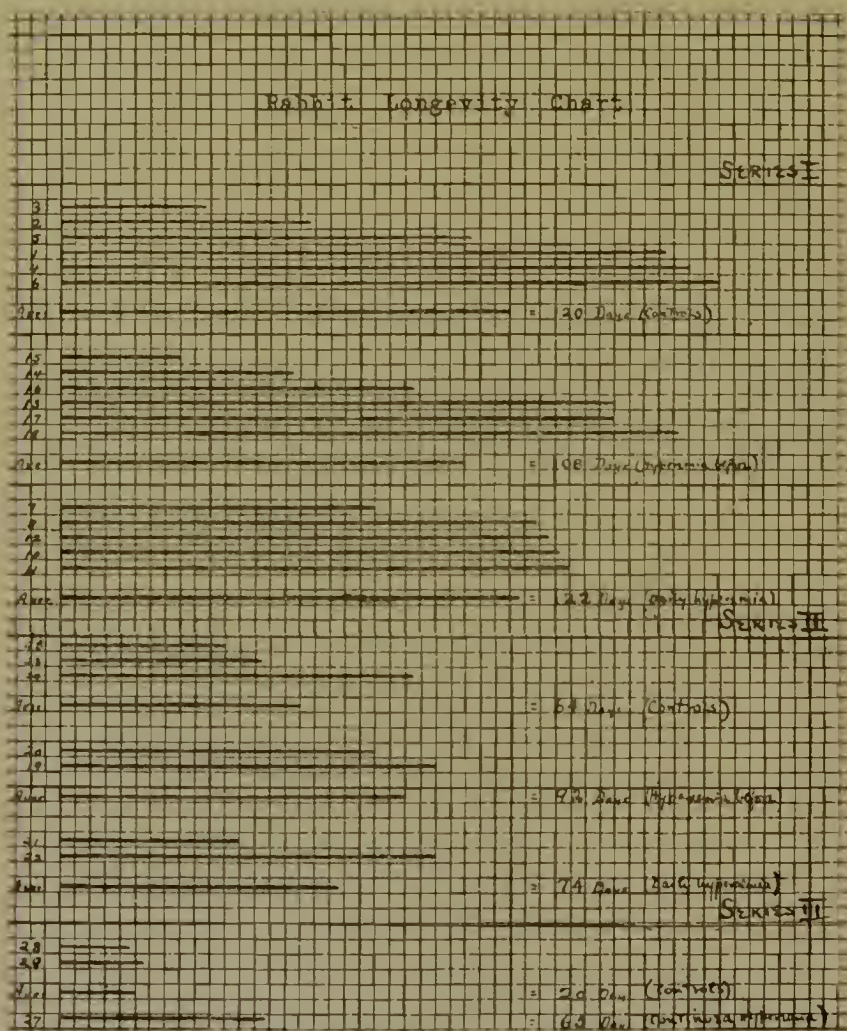
RABBIT	BODY SURFACE	LUNGS	LIVER	SPLEEN	KIDNEYS	PERITONEUM	MESENTERIC GLANDS	INGUINAL GLANDS
1	Large abscess left chest-wall.	+	+	+	+			+
2	Large abscess whole of both sides chest....	+	+	+	+			+
3	Ulcers over buttocks. Indurated areas both sides chest and abdomen	+	+			+		+
4	Large abscess area left groin.	+	Amyloid	Amyloid	Amyloid left +	+		+
5		+	+	+				+
6		+	+	+				+
7		+	+	+	+	+		+
8		+	+	+	+		+	+
9	Uterus completely inverted and protruding following a litter.	Small discrete tubercles						
10		+			+			
11		+	+		+			+
12		+	+	+	+			+
13		+	+	+	+			+
14		+	+	+	+			+
15	Left eye destroyed. Pus also from right.	+		+	+			+
16		+	+	+	+	+	+	
17		+		+	+	+		+
18		+			+			

Explanation of signs: + A few tubercles. ++ Moderate tuberculous condition.
+++ Advanced and excessive tuberculosis.

Reviewing the autopsy records, it seems curious that four out of the six controls should develop extensive lesions on the body surface, whereas no lesions of this kind occurred in the hyperemia group.

The following red blood-corpuscle averages indicate that the marrow hyperemia also causes an increase in the production of erythrocytes:

Normals—6 counts on 6 rabbits..... Average, 6,300,000
Normals—4 rabbits after one-half hour hyperemia..... “ 8,100,000



INFECTED RABBITS. BLOOD COUNTED SIX WEEKS AFTER INOCULATION

Controls, R. 1, 2, 4, 5, and 6.....	Average, 6,150,000
Hyperemias, R. 7, 8, 10, 11, and 12.....	" 9,400,000
Hyperemia before, R. 13, 14, 16, 17, and 18.....	" 8,100,000

SERIES II. RABBITS 19 TO 25

In this series the rabbits treated with hyperemia were set up in stocks during the application of the rubber to their hind legs. It was thought that by this position better and more certain hyperemia would be provoked. After the first few days one hour was found to be too much for their strength, and the time was shortened to half an hour.

Blood examinations were made daily before and after hyperemia, in rabbits 21 and 22 and the results are shown in charts.

SERIES III. RABBITS 26 TO 31

Rabbits 26 and 27 received continuous hyperemia following the inoculation of the standard dose of bovine tubercle bacilli. Average length of life, sixty-five days.

R. 26, weight.....	2674 grams
R. 27, weight.....	2790 "

Rabbits 28 and 29 served as controls. Average length of life, twenty days.

R. 28, weight.....	1510 grams
R. 29, weight.....	1550 "

Rabbits 30 and 31 received no inoculation of tubercle bacilli, but served as controls, with only the application of the rubber bands.

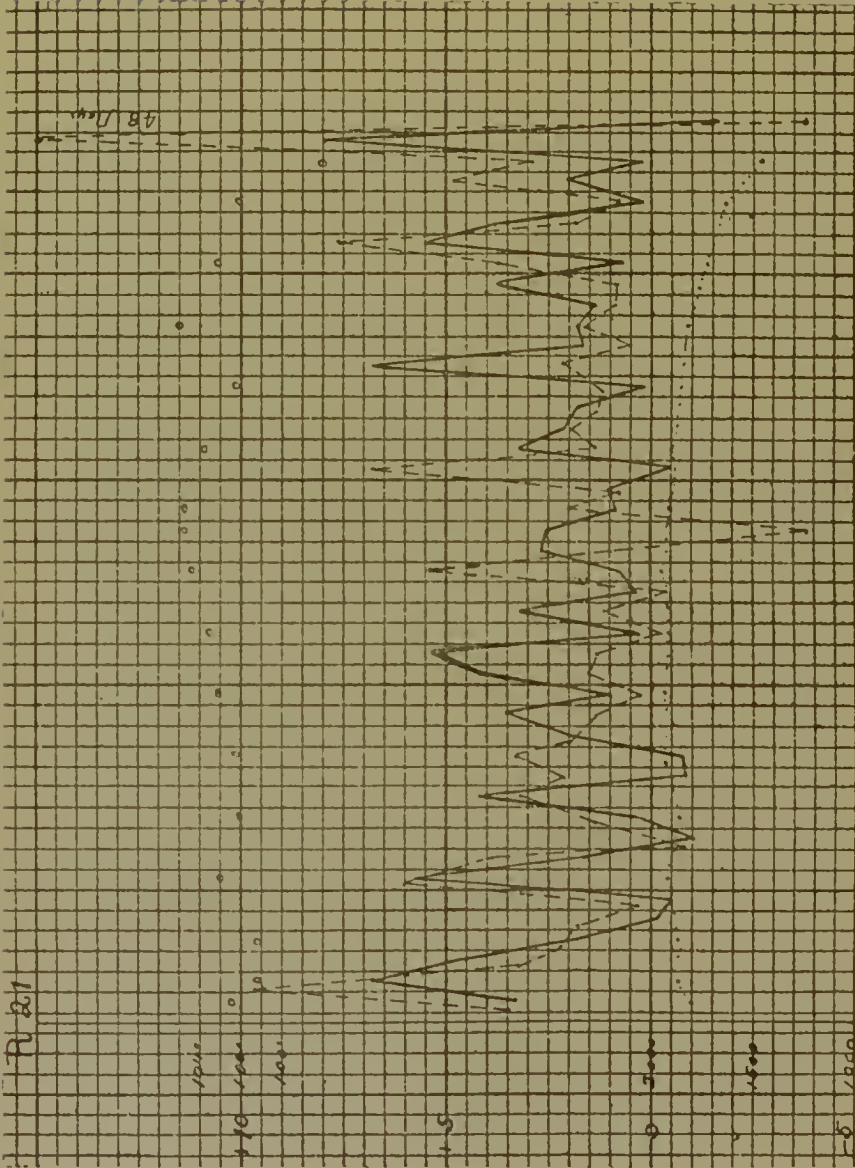
Rabbits 26, 27, 30, and 31 had applied to their forelegs rubber bands circled once around above the shoulders, and the loops then hooked together over the shoulders with a wire, which could be adjusted to tighten and loosen as necessary.

Rabbits 26 and 28 were found dead the same morning.

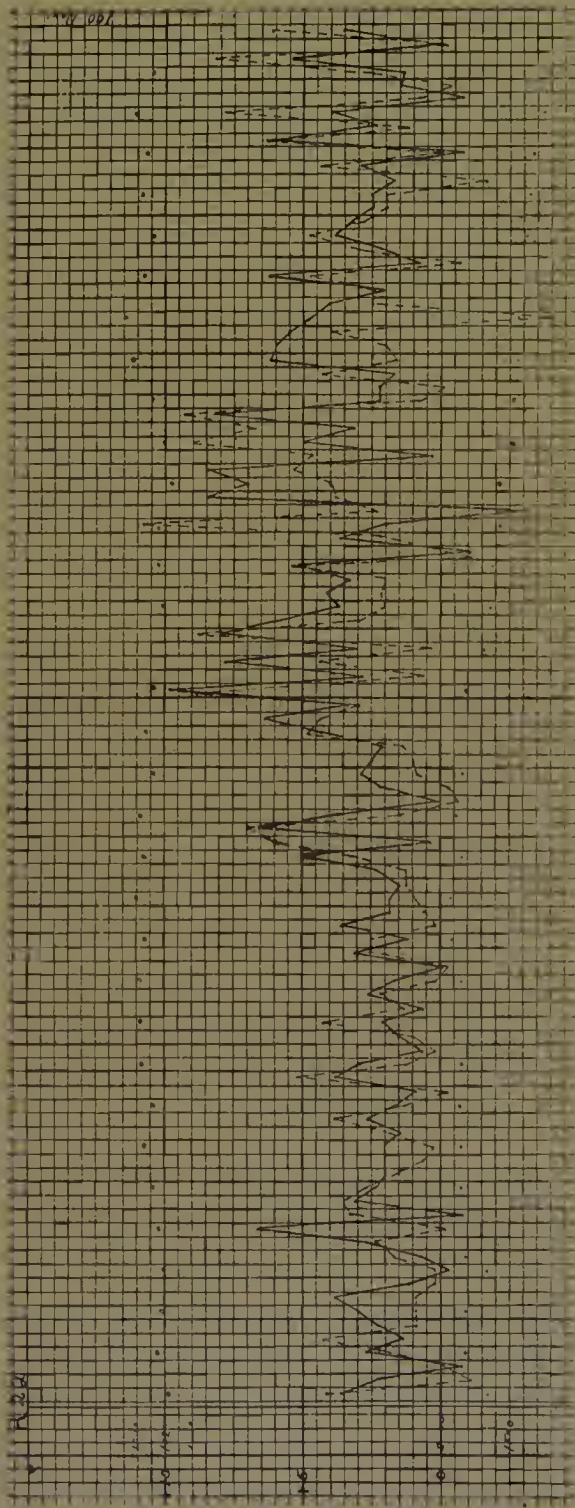
Rabbit 26 had accidentally hung himself to the wire netting of his cage by the wire hook on his back which held together the rubber bands on the legs.

His death was quite unfortunate, yet the contrast between the tubercle condition in the lungs and in those of rabbit 28 was most striking, and decidedly in favor of the continued hyperemia treated rabbit. The autopsy on rabbit 27 is as follows:

Rubber bands in place on upper part of forelegs. Beneath the bands the skin is eroded, and in several places the skin is entirely cut through—no evi-



The chart represents the changes produced among the leukocytes by one-half hour hyperemia, expressed in total numbers of mononuclears (—) and polymorphonuclears (---) per c.mm. Each space corresponds to one day. For example, on first day, the mononuclears increased 3300 and the polymorphonuclears 3500; on sixth day, mononuclears decreased 500, and the polymorphonuclears increased 300.



Explanation same as Chart R. 21. It is of interest to note that in only 16 of the 144 counts (11 per cent.) included in R. 21 and R. 22 did the hyperemia fail to cause an increase in the number of mononuclears.

dence of any inflammation. The deep veins distal to bands are three or four diameters larger than those proximal.

Heart, liver, spleen, and mesenteric and inguinal glands free from tubercles. Throughout both kidneys, scattered whitish areas.

Peritoneum smooth and glistening. Intestinal mucosa reddened and contents semifluid and brownish.

Marrow of femur examined macroscopically and compared with that of forelegs, but no difference was noted.

The mononuclear and polymorphonuclear leukocytes varied as follows:

	MONONUCLEARS	POLYMORPHONUCLEARS
RABBIT 26:		
Before infection.....	15,162 per c.mm.	2,473 per c.mm.
After 7 days continued hyperemia.	8,095 "	2,133 "
" 14 " " "	4,831 "	2,546 "
RABBIT 27:		
Before infection.....	9,868 "	3,947 "
After 7 days continued hyperemia.	7,114 "	968 "
" 14 " " "	6,947 "	3,422 "
" 21 " " "	6,412 "	4,980 "
" 28 " " "	7,484 "	4,539 "
" 35 " " "	6,145 "	5,719 "
" 42 " " "	6,400 "	7,708 "
" 49 " " "	8,242 "	7,300 "
" 56 " " "	7,728 "	8,904 "
RABBIT 28:		
Before infection.....	7,568 "	8,448 "
After 8 days continued hyperemia.	5,558 "	3,532 "
" 19 " " "	4,000 "	1,664 "
RABBIT 29:		
Before infection.....	5,584 "	5,774 "
After 8 days continued hyperemia.	5,177 "	2,588 "
" 19 " " "	29,607 "	30,583 "
RABBIT 30:		
Not infected.....	4,723 "	2,546 "
After 7 days continued hyperemia.	3,993 "	11,510 "
" 14 " " "	11,404 "	5,875 "
" 21 " " "	5,599 "	4,562 "
" 28 " " "	9,679 "	2,756 "
RABBIT 31:		
Not infected.....	7,168 "	3,976 "
After 7 days continued hyperemia.	8,193 "	2,958 "
" 14 " " "	7,413 "	4,984 "
" 21 " " "	5,100 "	6,240 "

The marrow considered as an organ has great capacity for increase in territory when actively called upon for the production of more blood-corpuscles. The fatty packing and the cancellous bone can readily be removed, and their

space occupied by proliferating bone-marrow cells when the appropriate stimulus is received.

"Various theories have been advanced to explain the production of inflammatory leukocytosis or the transitory increase of certain of the white cells, occurring in the blood in most of the acute infective diseases, and in other similar conditions. Various chemical substances in solution have been found to attract or to repel certain of the protozoa and to this phenomenon Pfeiffer gave the name chemiotaxis—positive or negative according as the protozoa approach or avoid the substance employed. Pfeiffer also found that bacteria can be similarly influenced by the chemical constituents of nutritive substances, and he and numerous other observers have established the fact that leukocytes can in like manner be influenced by certain bacterial and chemical bodies, positively or negatively as the case may be. This so-called 'chemiotactic action' of bacteria and their products upon leukocytes was found by Leber to be capable of being exerted at a distance, and thus the presence of increased numbers of leukocytes in the circulating blood came to be regarded as due to their being attracted or drawn from the blood-forming organs by positive chemiotaxis; while in the leukopenia or absence of leukocytosis found in typhoid fever and in measles the bacterial products are supposed to exercise a negative or repellant action upon the cells. It is not my intention here to discuss the question of how this increased output of blood elements by the bone-marrow is brought about, except in so far as an explanation may be found in the proliferative and other changes in the tissue which accompany it. Whether this proliferation and increased output of cells are due to direct stimulation of the marrow itself, or are merely a regenerative effort on the part of the tissue in order to replace the cells which have been withdrawn from it, it is extremely difficult to determine; and, again, the question whether the chemiotactic substances bringing about these processes in the marrow are produced by the bacteria themselves, or are derived from the leukocytes destroyed by them, is a problem of considerable difficulty, and it is not unlikely that all the factors mentioned may assist in varying degree in the production of these phenomena.

"Whatever be the nature of the cause of this reaction in the blood-forming tissues, the series of phenomena which accompany, or rather which comprise, the actual process, usually follow a somewhat definite order, and the first phenomenon to appear, whether in marrow which is already red in type or in fatty marrow which is about to be transformed into such active hematopoietic tissue, is an *increased supply of blood to the part*"—Dickson.¹¹

It is perhaps, therefore, conceivable that by forcing more blood into marrow

which is inert or suffering from a negative chemiotactic condition, valuable leukoblastic results might be obtained.

However, it must not be forgotten that in many cases of pulmonary tuberculosis a condition of the marrow occurs known as mucoid transformation, which allows of no leukoblastic reaction.

We have applied this principle of marrow hyperemia to some thirty pulmonary patients, nearly all, however, in advanced conditions, when it is more than probable the marrow has been exhausted.

Even in these patients, as shown above, it is possible to completely change the blood-picture.

In three comparatively early cases, however, marked improvement took place. Weight was rapidly gained and sputum disappeared.

Further reports of the application to man will, however, be made later.

If the tourniquet principle be applied, the following precautions must be observed:

- (1) The best time for the application is daily at bedtime.
- (2) Ten minutes should be the duration of the first treatment, followed by twenty, thirty, forty, and fifty minutes, up to one hour, if the patient can stand it.
- (3) Patients must be cautioned never to fall asleep with bandage on.
- (4) It is wise to have a second person present during the application.
- (5) The rubber bandage should be removed from first one leg and then from the other.

The continued form of hyperemia as described above is probably the better and the application to arms and legs can be alternated.

CONCLUSIONS.

1. It would appear that rabbits at this altitude, which possess 69 per cent. of lymphocytes as against 52 per cent. at sea-level (Brinkerhoff and Tyzzer¹⁴), live longer than sea-level rabbits, following a standardized lethal dose of virulent bovine tubercle bacilli. This occurrence may help to explain the rarity of human tuberculosis manifesting itself here.

2. Artificial marrow hyperemia seems to produce a genuine lymphocytosis, and this occurrence would corroborate the idea that it is the marrow hyperemia of altitude which causes the increase of lymphocytes noted by us at this elevation.

3. Artificial marrow hyperemia, by producing a lymphocytosis and some leukocytosis, appears to hold in check a rapid bovine tuberculous process in rabbits.

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